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Vascular hyporesponsiveness in simulated microgravity: role of nitric oxide-dependent mechanisms

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Sangha, D. Sara, N. D. Vaziri, Y. Ding, and R. E. Purdy. Vascular hyporesponsiveness in simulated microgravity: role of nitric oxide-dependent mechanisms. *J. Appl. Physiol.* 88: 507–517, 2000.—Simulated microgravity depresses the ability of arteries to constrict to norepinephrine (NE). In the present study the role of nitric oxide-dependent mechanisms on the vascular hyporesponsiveness to NE was investigated in peripheral arteries of the rat after 20 days of hindlimb unweighting (HU). Blood vessels from control rats and rats subjected to HU (HU rats) were cut into 3-mm rings and mounted in tissue baths for the measurement of isometric contraction. Mechanical removal of the endothelium from carotid artery rings, but not from aorta or femoral artery rings, of HU rats restored the contractile response to NE toward control. A 10-fold increase in sensitivity to ACh was observed in phenylephrine-precontracted carotid artery rings from HU rats. In the presence of the nitric oxide synthase (NOS) substrate L-arginine, the inducible NOS inhibitor aminoguanidine (AG) restored the contractile responses to NE to control levels in the femoral, but not carotid, artery rings from HU rats. In vivo blood pressure measurements revealed that the peak blood pressure increase to NE was significantly greater in the control than in the HU rats, but that to AG was less than one-half in control compared with HU rats. These results indicate that the endothelial vasodilator mechanisms may be upregulated in the carotid artery, whereas the inducible NOS expression/activity may be increased in the femoral artery from HU rats. These HU-mediated changes could produce a sustained elevation of vascular nitric oxide levels that, in turn, could contribute to the vascular hyporesponsiveness to NE.

rats; blood vessels; norepinephrine; in vivo

UNDER THE INFLUENCE of gravity, tissue fluids are shifted toward the lower half of the body in standing humans. This is reflected by a blood pressure gradient that is 70 mmHg at the head and 200 mmHg at the feet (12). When humans are exposed to microgravity during spaceflight, fluids shift toward the head and the pressure gradient is eliminated, becoming 100 mmHg throughout. This marked hemodynamic change occurs rapidly on exposure to microgravity and could be the triggering event for subsequent cardiovascular adaptations.

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As reviewed by Watenpaugh and Hargens (36), the changes induced by microgravity include an initial variable increase, then a chronic decrease, in central venous pressure. Hypovolemia also occurs as a result of reductions in plasma and extracellular fluid volume and red blood cell mass. Several authors have suggested that the cardiac baroreceptor reflex response to gravitational challenge is impaired (7, 31), as may be the vasoconstrictor component of the baroreceptor reflex (3, 19). These and other changes may be appropriate for the microgravitational environment. However, they become inappropriate on reexposure to gravity. The most serious consequence experienced by microgravity-adapted astronauts on return to Earth is the development of postural intolerance. Namely, these individuals are at risk of syncope. In this light, the adaptations induced by microgravity are referred to as "cardiovascular deconditioning."

A recent study by Buckey and co-workers (3) characterized hemodynamic parameters in 14 astronauts. As a part of that study, the astronauts were subjected to stand tests before and after spaceflight. Nine astronauts were not able to complete the test after spaceflight because of postural intolerance. When these nine astronauts were compared with those who finished the stand test, the only significant difference was a "greater postflight vasoconstrictor response with higher total peripheral resistance during standing in the finishers." This study points to the importance of vascular changes in microgravity and how these changes influence of cardiovascular deconditioning on gravitational challenge.

Direct evidence for reduced vascular responsiveness has come from animal studies using real and simulated microgravity. Sayette and co-workers (27) found that the sensitivity of the vena cava to norepinephrine (NE) was reduced in rats exposed to microgravity during spaceflight or simulated microgravity in the form of hindlimb unweighting (HU). Delp and co-workers (5, 6) found that the maximal contractile response of the isolated rat aorta to several vasoconstrictor agents was reduced by HU. In addition, the present authors recently reported that HU reduced vascular contractility in a range of blood vessels (25).

The purpose of the present study was to explore possible mechanisms underlying the vascular hyporesponsiveness induced by simulated microgravity. Hypothetically, decreased vascular contractility could result from impairment of contractile mechanisms and/or

enhancement of vasodilator mechanisms. In a preliminary study (26) the present authors found evidence for increased activity of nitric oxide-dependent processes in certain blood vessels from rats subjected to HU (HU rats). The present study was carried out to characterize these processes further. Our findings are consistent with the possibility that HU increased endothelium-dependent and inducible nitric oxide synthase (iNOS)-dependent vasodilator activities.

METHODS

The Institutional Animal Care and Use Committee of the University of California, Irvine, approved animal procedures. Male Wistar rats weighing 250–300 g were obtained from Simonsen Laboratories (Gilroy, CA) and housed in a temperature-controlled room (22°C) with a 12:12-h light-dark cycle. Water and rat chow were provided ad libitum. Animals were randomly assigned to control (C) or HU groups. HU was achieved by using a tail harness to partially elevate the hindlimbs of the animals above the floor of the cage by a modification (25) of the method of Thomason et al. (32). Briefly, the tail was cleaned, a coat of tincture of benzoin was applied, and the tail was air dried until tacky. Adhesive strips (Fas-Trac Company of California, Van Nuys, CA), the width of the tail, were then looped through a swivel harness and pressed along the sides of the tail to form a tubular casing around the tail. Thereafter, the tail was wrapped first with Elastoplast bandage (Beiersdorf, Norwalk, CT) and then with a thin layer of plaster cast material (Sammons Preston, Bolingbrook, IL). The rat was suspended by the swivel harness from a hook at the top center of the suspension cage, which allowed free 360° rotation. The height of the hook was adjusted such that the front limbs were in contact with the floor and the hindlimbs were elevated ~0.5 cm above the floor, tilting the body of the rat to an angle of 35° with the floor of the cage. The animals were subjected to HU for 20 days, and then the HU animal and the paired control were used in the following *in vitro* experiments.

Artery ring preparation. HU and control rats were euthanized by exposure to 100% CO₂ for 90 s to induce deep anesthesia (10). The chest was opened, and the heart was removed. The abdominal aorta and the femoral and carotid arteries were collected and placed in warm oxygenated Krebs bicarbonate solution. The tissues were cleaned under the microscope to remove extraneous fatty and connective tissue and then cut into 3-mm rings. In protocols examining responses in the absence of vascular endothelium, the endothelium was removed from the abdominal aorta by inserting 3-0 silk suture into the lumen and rolling the interior surface of the artery ring against the suture. This was accomplished by using the suture to pull the ring gently back and forth on moistened filter paper. Two strands of 32-gauge stainless steel wire were tightly twisted to form a single 5-cm-long wire bundle. This bundle fitted easily into the lumens of the carotid and femoral artery rings and was used in the place of the 3-0 silk suture to remove the endothelium from these vessels. Success of the endothelium removal was confirmed by the loss of ACh-mediated relaxation of phenylephrine-precontracted vessels.

***In vitro* isometric contraction experiments.** Abdominal aorta and carotid and femoral artery rings were mounted in tissue baths for the measurement of isometric contraction, and they were stretched to optimal resting forces, previously determined in an earlier study from our laboratory (25). In that study (25), HU had no effect on dry or wet weights of vessel rings. In addition, in subsequent studies (14), HU reduced the

contractile response to NE but not to serotonin. This implies that the effect of HU was not mediated by possible vascular smooth muscle remodeling or vessel atrophy and validates our use of previously determined optimal resting forces: 2 g for aorta, 1.5 g for control carotid artery, 1 g for HU carotid artery, and 1 g control and HU femoral artery. Vessel rings were equilibrated for 30 min in 37°C, oxygenated Krebs bicarbonate solution of the following composition (in mM): 119.2 NaCl, 4.9 KCl, 1.3 CaCl₂, 1.2 MgSO₄, 25 NaHCO₃, 11.1 glucose, 0.114 ascorbic acid, and 0.03 tetrasodium ethylenediaminetetraacetate. Subsequently, the artery rings were contracted with Krebs solution containing 68 mM K⁺ prepared by equimolar replacement of Na⁺. When the tissues had reached steady-state contraction, they were washed twice with normal Krebs solution and allowed to relax to resting levels. Contractions with 68 mM K⁺ were repeated 20–30 min later. Preliminary experiments revealed that all tissues yielded uniform magnitudes of contraction to the second and all subsequent exposures to 68 mM K⁺. After the tissues recovered from the second exposure to 68 mM K⁺, agonist concentration-response curves (CRCs) were obtained by the cumulative addition of agonists in 0.5-log increments. When NE was used, 30 μM cocaine and 30 μM deoxycorticosterone acetate were added to the bathing medium 30 min before the CRC was obtained to block neuronal (8) and extraneuronal (13, 15) catecholamine uptake and 1 μM propranolol was added to block β-adrenoceptors (1). In some experiments, 0.3 μM L-arginine and 100 μM aminoguanidine were added to the bathing medium 30 min before the NE CRC. Neither of these agents had any effect on vessel resting force.

***In vitro* isometric relaxation experiments.** To study the relaxing effects of ACh and sodium nitroprusside, the following protocol was used. A phenylephrine CRC was obtained to precontract the tissue. Subsequently, an ACh relaxation CRC was obtained. Tissues were then washed twice with Krebs solution and allowed to equilibrate at resting force for 30 min. A second phenylephrine CRC was obtained and, at the highest concentration of phenylephrine used (300 μM), 10 μM *N*-nitro-L-arginine methyl ester (L-NAME) was added to block nitric oxide synthase (NOS). The tissues typically contracted further in the presence of L-NAME. When the tissues had reached a new steady-state contraction, sodium nitroprusside relaxation CRC was obtained. In experiments to test the effect of the iNOS-selective inhibitor aminoguanidine on ACh- or L-arginine-induced relaxations, one-half of the artery rings were exposed to 100 μM aminoguanidine 30 min before precontraction of all artery rings with 10 μM NE. When the NE contraction was at steady state, ACh relaxation curves (0.01–30 μM) were obtained or the artery rings were exposed to 0.3 or 1 μM L-arginine. In these experiments the relaxation responses were expressed as a percentage of phenylephrine or NE precontraction. The contractile response to the agonists was taken as a 100% contraction, and the relaxation response was measured as a percentage of that contraction. This was done to normalize relaxation responses, since the contractile responses to the agonists were different between the HU and control tissues.

***In vivo* blood pressure measurements.** Under general anesthesia with thiobutabarbital (Inactin, 100 mg/kg ip), the left jugular vein and carotid artery were cannulated with polyethylene (PE-50) tubes. A tracheal cannula was then inserted, and the animal was placed on a heating pad. Arterial blood pressure was monitored directly via the arterial catheter, which was connected to a Gould P-50 pressure transducer, and recorded on a Dynograph R511A recorder (Sensor Medics, Anaheim, CA). Once stable, the blood pressure was continuously recorded for 5 min to determine the baseline value.

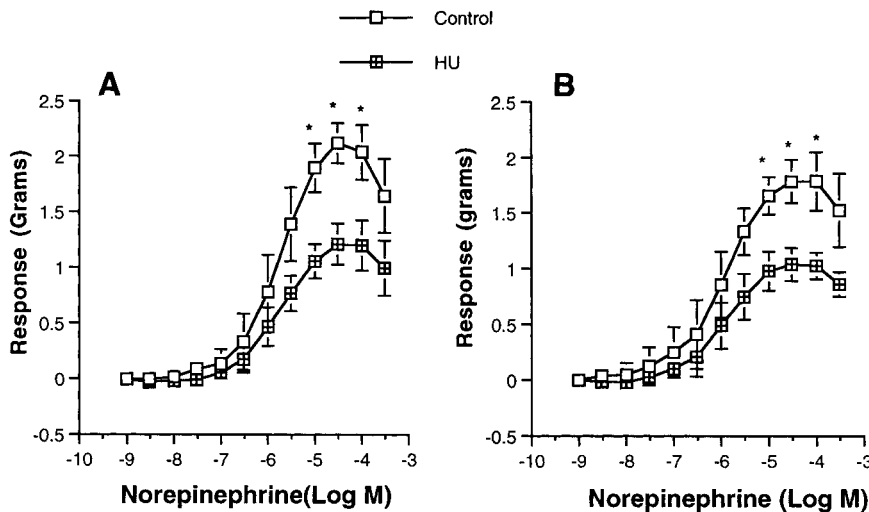


Fig. 1. Concentration-response curves for contractile effect of norepinephrine (NE) in abdominal aorta rings from control and 20-day hindlimb-unweighted (HU) rats. Endothelium was mechanically removed from 2 of 4 tissue rings from each animal. A: endothelium-intact rings; B: endothelium-denuded rings. Values are means \pm SE; $n = 5$ rats. * $P < 0.05$.

Subsequently, pressor responses to bolus injections of NE (0.15 $\mu\text{g/kg}$; Sigma Chemical, St. Louis, MO) and the iNOS inhibitor aminoguanidine (30 mg/kg; Sigma Chemical) were determined. The response to each drug was calculated as peak change in blood pressure from the baseline value. Mean arterial pressure (MAP) was calculated as the sum of diastolic blood pressure and one-third of the pulse pressure. Each drug was injected at least twice, and the average of the values was used. A 30-min recovery period was allowed after each bolus injection of NE. The aminoguanidine injections were separated by a 60-min interval.

Isometric contractions and relaxations were recorded using force transducers (model FT03C, Grass Instruments, Quincy, MA, or Fort 10 Load Cell, World Precision Instruments, Sarasota, FL) connected to MacLab electronic data acquisition systems (Castle Hill, Australia). All agents were added to the bathing medium in volumes of $\leq 100 \mu\text{l}$. NE, phenylephrine, ACh, and sodium nitroprusside solutions were prepared fresh each day, and stock solutions of cocaine, deoxycorticosterone acetate, propranolol, and L-NAME were prepared weekly and maintained at 4°C. Deoxycorticosterone acetate was dissolved in 50% ethanol and all other drugs in double-distilled water.

The number of animals used for each CRC is indicated by n . Four rings of each type of blood vessel were obtained from each animal. Thus two control and two treated rings from control and HU rats were used in each experiment. Contraction

responses to drugs are presented as absolute values in grams force development. Vasodilator responses are presented as percent relaxation from precontracted levels. CRCs were compared by repeated-measures, two-way ANOVA with SuperANOVA software (Abacus Concepts, Berkeley, CA), and differences between individual points on different CRCs were analyzed by post hoc Scheffé's test. $P < 0.05$ was used as the criterion for significance.

RESULTS

Endothelium-dependent mechanisms. The purpose of the present study was to assess the possible contribution of the endothelium, and of nitric oxide-dependent mechanisms, to the HU-induced vascular hyporesponsiveness to NE. The first approach to this question was to mechanically remove the endothelium. In the abdominal aorta, HU resulted in a significantly reduced response to NE in endothelium-intact and -denuded vessel rings (Fig. 1). Thus the depression of contractility caused by HU appeared to be independent of the endothelium in the aorta. In the carotid artery, HU depressed the contraction to NE in endothelium-intact vessel rings (Fig. 2). In contrast, the maximal response of endothelium-denuded HU vessels to NE approached, and was not significantly different from, that of de-

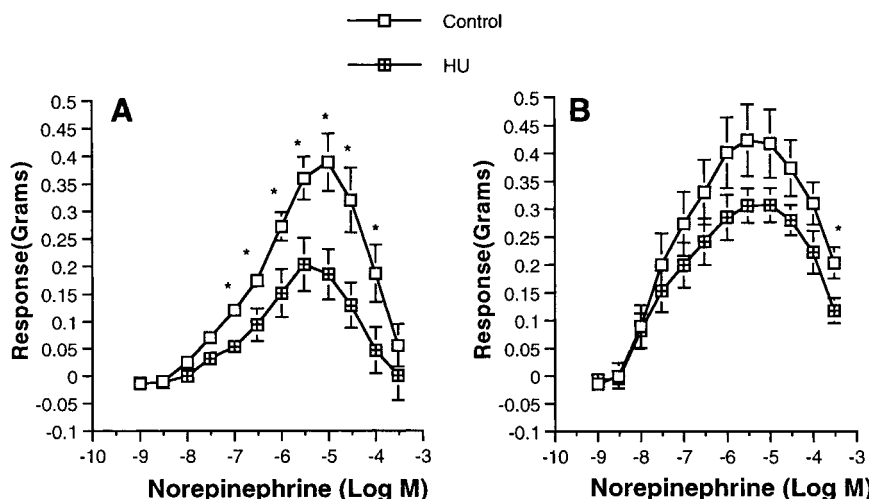
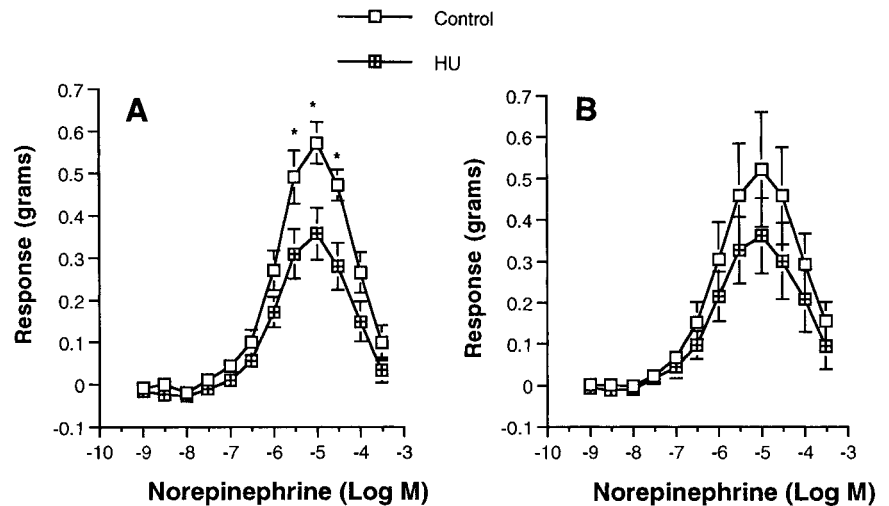


Fig. 2. Concentration-response curves for contractile effect of NE in carotid artery rings from control and 20-day HU rats. Endothelium was mechanically removed from 2 of 4 tissue rings from each animal. A: endothelium-intact rings; B: endothelium-denuded rings. Values are means \pm SE; $n = 5$ rats. * $P < 0.05$.

Fig. 3. Concentration-response curves for contractile effect of NE in femoral artery rings from control and 20-day HU rats. Endothelium was mechanically removed from 2 of 4 tissue rings from each animal. *A*: endothelium-intact rings; *B*: endothelium-denuded rings. Values are means \pm SE; $n = 8$ rats. * $P < 0.05$.



nuded control vessels. In the femoral artery, endothelium removal caused a small, insignificant reduction in the difference between control and HU maximal contractions to NE (Fig. 3) but also markedly increased the variability of contractile responses, as indicated by larger standard error bars, compared with those in the endothelium-intact tissues. The rat femoral artery is a relatively small, thin-walled vessel, and even gentle mechanical removal of the endothelium may have caused various levels of damage to the underlying smooth muscle cells. Power calculations revealed that there were insufficient data, even with eight experiments in duplicate, to determine whether endothelium removal had any significant effect. Other experimental approaches are required to determine the possible contribution of the endothelium to the HU effect in the femoral artery.

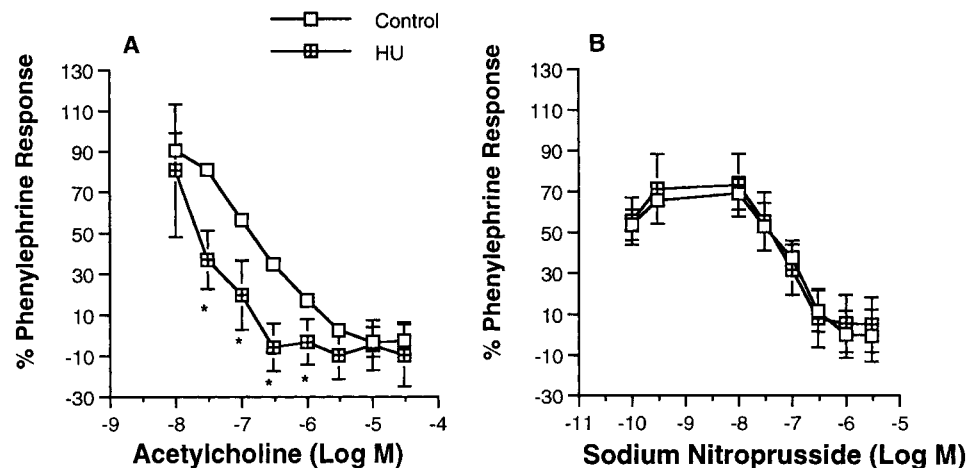
The finding that endothelium removal increased the contractile response of HU carotid arteries to NE toward control implied that HU induced a change in endothelial function in this vessel. To explore this effect further, control and HU carotid arteries were precontracted with phenylephrine, and ACh relaxation CRCs were obtained. ACh causes relaxation by stimulating the synthesis and release of nitric oxide from the

endothelium (29). As shown in Fig. 4A, ACh relaxed control and HU vessels completely. However, the HU vessels were more sensitive to the relaxing effects of ACh; i.e., the ACh CRC in HU vessels was shifted to the left ~ 10 -fold compared with control. Subsequently, relaxation curves to sodium nitroprusside were also obtained in the same vessels, precontracted a second time with phenylephrine, and the results are shown in Fig. 4B. Again, all vessels relaxed completely. However, there was no difference in the sensitivities of control and HU vessels to this endothelium-independent relaxing agent.

Similar experiments were carried out in the femoral artery, and the results are shown in Fig. 5. ACh and sodium nitroprusside caused complete relaxation of precontracted control and HU vessels. However, in contrast to the carotid artery, there were no differences in sensitivities between control and HU femoral arteries to either relaxing agent.

The results of relaxation experiments carried out in the abdominal aorta closely resembled those in the femoral artery (Fig. 6). In general, there were no differences between control and HU vessels in response to ACh or sodium nitroprusside. One exception was

Fig. 4. Concentration-response curves for relaxing effects of ACh (*A*) and sodium nitroprusside (*B*) in phenylephrine-precontracted carotid artery rings from control and 20-day HU rats. Values are means \pm SE; $n = 4$ rats. * $P < 0.05$.



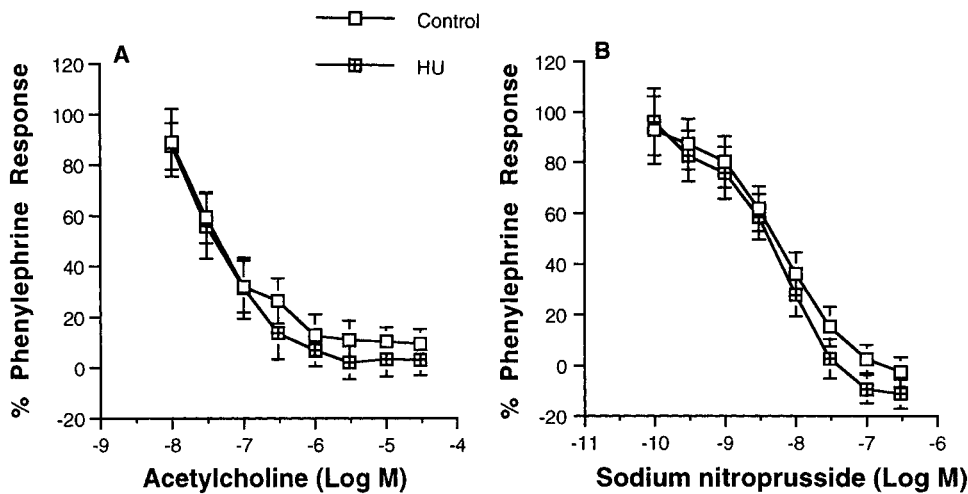


Fig. 5. Concentration-response curves for relaxing effects of ACh (A) and sodium nitroprusside (B) in phenylephrine-precontracted femoral artery rings from control and 20-day HU rats. Values are means \pm SE; $n = 5$ rats. * $P < 0.05$.

that the aorta rings subjected to HU relaxed significantly less at 1 nM sodium nitroprusside (Fig. 6B).

iNOS-dependent mechanisms. To explore the possible contribution of iNOS to the vascular hyposensitivity to NE in HU tissues, the selective iNOS inhibitor aminoguanidine (37) was used. Initial experiments were conducted to demonstrate the iNOS selectivity of aminoguanidine. Control and HU carotid and femoral arteries were precontracted with NE, and ACh relaxation CRCs were obtained in the presence and absence of 100 μ M aminoguanidine. As shown in Fig. 7, aminoguanidine had no effect on the ACh relaxation response. Because ACh causes relaxation by activating endothelial constitutive NOS (eNOS), leading to nitric oxide production (22), these results indicate that aminoguanidine had no effect on eNOS.

It has been shown recently that iNOS exists constitutively in vascular and nonvascular tissues of the rat and exhibits altered expression in various disease states (35). In addition, iNOS synthesizes nitric oxide continuously as long as the substrate L-arginine is available (20). To test the ability of aminoguanidine to inhibit iNOS-dependent vascular smooth muscle relaxations, abdominal aorta and carotid and femoral artery

rings were precontracted with NE and exposed to 0.3 and 1 μ M L-arginine. As shown in Fig. 8A, in the abdominal aorta, L-arginine caused a slight concentration-dependent relaxation in HU and control vessels, and aminoguanidine had no significant effect on the L-arginine-induced relaxation. In the femoral artery (Fig. 8C), L-arginine caused significant concentration-dependent relaxations that were attenuated by aminoguanidine. The degree of relaxation caused by L-arginine and the degree of attenuation of relaxation by aminoguanidine was greater in the HU than in the control femoral artery rings. In the carotid artery (Fig. 8B), L-arginine caused concentration-dependent relaxations in control and HU tissue rings. The relaxation response induced by 0.3 μ M L-arginine was not significantly affected by aminoguanidine; however, aminoguanidine attenuated the relaxation response to 1 μ M L-arginine in HU, but not in control, carotid artery rings. These data suggest that HU results in upregulation of iNOS activity in rat femoral and carotid arteries, but the degree of increase in iNOS activity is greater in the femoral artery. Aminoguanidine had no effect on L-arginine relaxation in control or HU abdominal aorta,

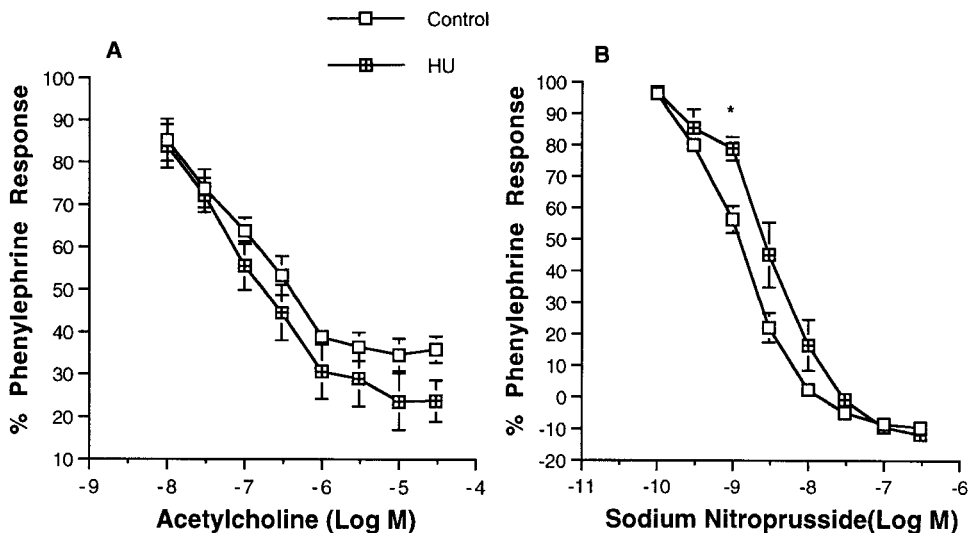


Fig. 6. Concentration-response curves for relaxing effects of ACh (A) and sodium nitroprusside (B) in phenylephrine-precontracted abdominal aorta rings from control and 20-day HU rats. Values are means \pm SE; $n = 5$ rats. * $P < 0.05$.

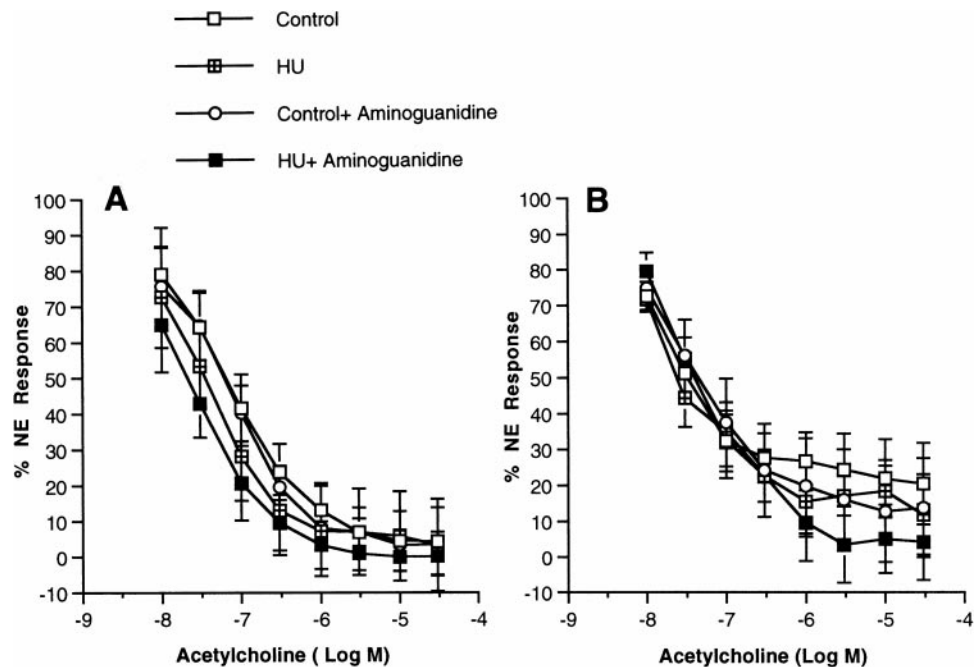


Fig. 7. Concentration-response curves for relaxing effects of ACh in NE-precontracted carotid (A) and femoral (B) artery rings with and without aminoguanidine (AG, 100 μ M) from control and 20-day HU rats. Values are means \pm SE; n = 5 rats.

indicating that iNOS does not play a role in the hyporesponsiveness to NE in the HU abdominal aorta.

As a further test of the contribution of iNOS to the HU-induced hyporesponsiveness to NE, NE CRCs were obtained in blood vessels in the presence and absence of aminoguanidine. Because the activity of iNOS is known to be substrate dependent, the CRCs were carried out in the presence of 0.3 μ M L-arginine. Aminoguanidine had no effect on the NE CRCs in control or HU abdominal aorta or carotid artery rings (Fig. 9, A and B). In the femoral artery (Fig. 9C), aminoguanidine had no effect on NE CRCs in control rings. However, aminoguanidine markedly and significantly increased the contractile response to NE in HU femoral artery rings. Moreover, the NE CRC in the HU femoral artery rings in the presence of aminoguanidine was not significantly different from the NE CRC in the control artery rings; i.e., in the presence of L-arginine, aminoguanidine completely reversed the HU-induced hyporesponsiveness to NE in the femoral artery.

NE CRCs were also obtained in control and HU carotid and femoral artery rings in the presence and absence of aminoguanidine but in the absence of L-arginine to evaluate the dependence of iNOS activity on the availability of endogenous substrate. Aminoguanidine had no effect on control carotid and femoral artery responses to NE but caused a slight increase in the NE response in HU carotid and femoral artery rings (Fig. 10).

In vivo blood pressure measurements. The in vitro results with aminoguanidine in the carotid and femoral arteries raised the possibility that HU could increase the activity and/or the expression of vascular iNOS. Such a change could influence blood pressure regulation in vivo. To test this possibility, control and HU rats were instrumented with arterial and venous cannulas to allow blood pressure measurement and drug injection,

respectively. Peak pressor responses to NE and aminoguanidine are shown in Fig. 11. The peak blood pressure increase to NE was significantly greater in control than in HU rats: 50 and 28 mmHg, respectively. In contrast, the blood pressure elevation elicited by aminoguanidine was significantly less in control than in HU rats: 25 and 55 mmHg, respectively.

DISCUSSION

Previous studies using the HU rat have shown that the vascular contractile response is impaired in this simulation model of microgravity. For example, the maximal contractile response to a variety of vasoconstrictor agents was reduced in the abdominal aorta (5, 6, 25) and carotid and femoral arteries (25) by HU.

Studies in the aorta have tended to rule out a contribution of endothelial mechanisms to the HU-induced vascular hyporesponsiveness. In Sprague-Dawley rats, endothelium removal did not prevent the HU effect (6), and the sensitivities of precontracted control and HU aorta rings to the relaxing effects of ACh or sodium nitroprusside were not different. Similar results were found in the present study in the aorta of Wistar rats. Namely, endothelium removal did not alter the HU-induced hyporesponsiveness to NE, and HU treatment had no effect on sensitivity to ACh. These results suggest that the HU effect on the response of isolated abdominal aorta rings to NE is mediated by endothelium-independent mechanisms.

In contrast to these results in Sprague-Dawley and Wistar rats, Delp and co-workers (5) found that the sensitivities of the HU aortas from Fischer 344/Brown Norway rats to ACh and sodium nitroprusside were reduced. Such decreases in sensitivity could signify that HU caused an elevated level of nitric oxide in vivo. In turn, this could have caused the decreased sensitiv-

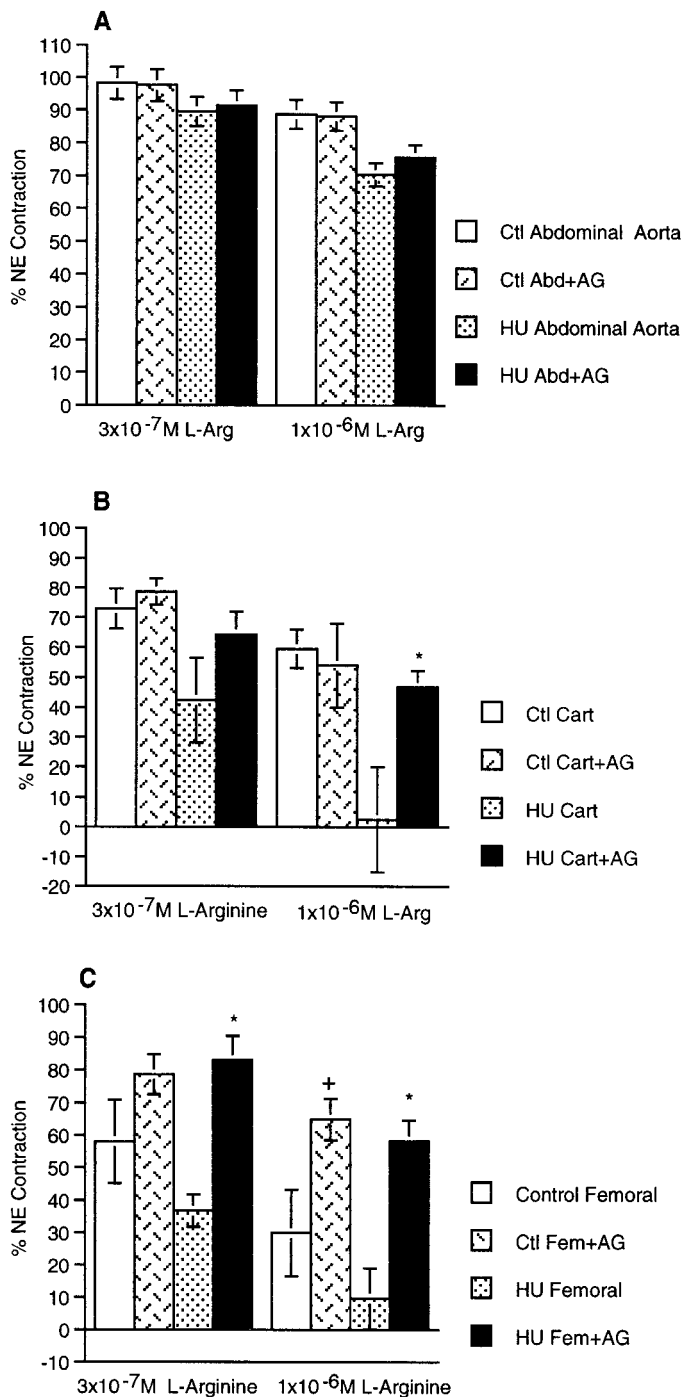


Fig. 8. Relaxing effects of 3×10^{-7} and 1×10^{-6} M L-arginine in abdominal aorta (A), carotid artery (B), and femoral artery (C) rings precontracted with NE, in presence and absence of AG. Artery rings are from control and HU rats, and relaxation is expressed as percentage of NE precontraction. Ctl, control; Abd, abdominal aorta; Cart, carotid artery; Fem, femoral artery. Values are means \pm SE; $n = 5$ rats. *HU different from Ctl + AG and HU + AG ($P < 0.05$); + Ctl different from Ctl + AG ($P < 0.05$).

ity to endogenous (ACh) or exogenous (sodium nitroprusside) agents observed in vitro. Nevertheless, Delp and co-workers reported that endothelium removal had no effect on the HU-induced hyporesponsiveness to NE in the aorta from Fischer 344/Brown Norway rats.

It is clear from the present results that the aorta is not representative of all blood vessels. Endothelium removal in the carotid artery substantially reversed the depression of contractility caused by HU (Fig. 2). Moreover, the HU carotid artery was markedly more sensitive than the control vessel to relaxing effects of ACh

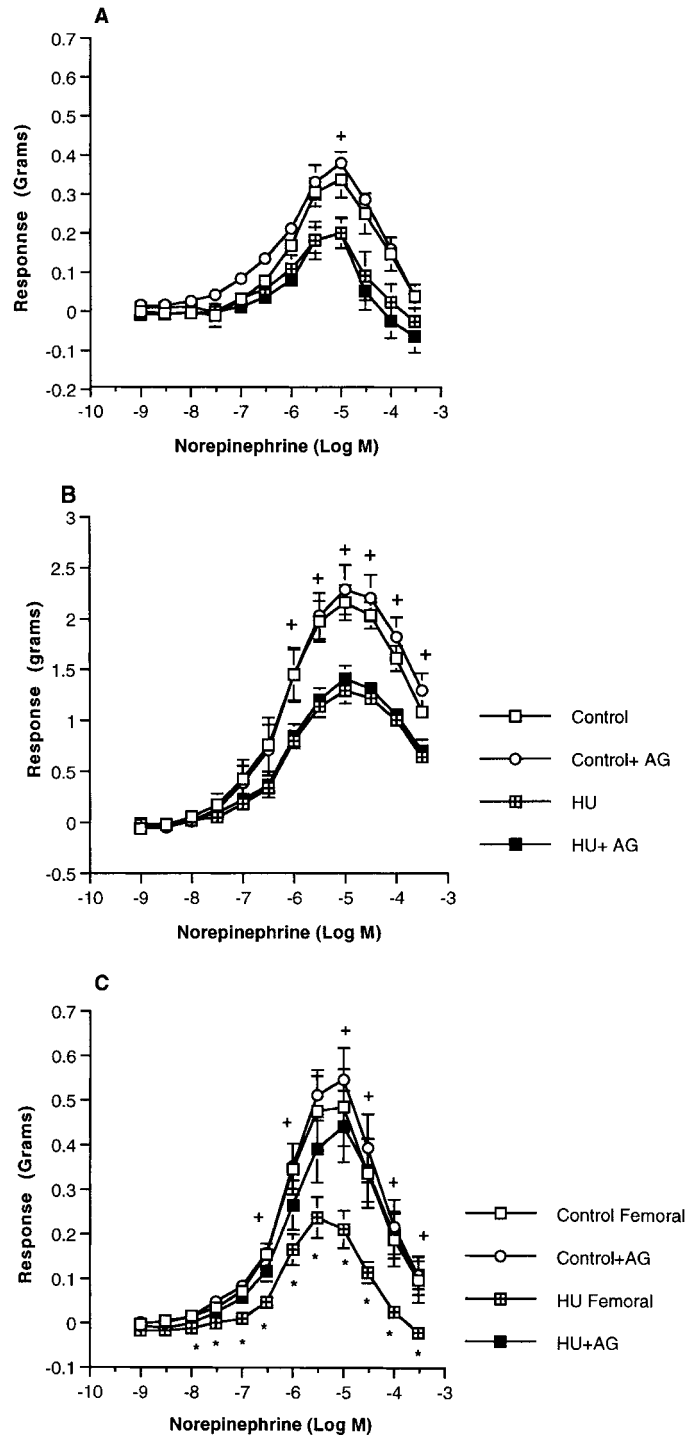


Fig. 9. Concentration-response curves for contractile effects of NE, in presence of L-arginine, in abdominal aorta (A), carotid artery (B), and femoral artery (C) rings with and without AG (1×10^{-4} M), from control and 20-day HU rats. Values are means \pm SE; $n = 5$ rats. *HU different from HU + AG ($P < 0.05$); + HU different from control ($P < 0.05$).

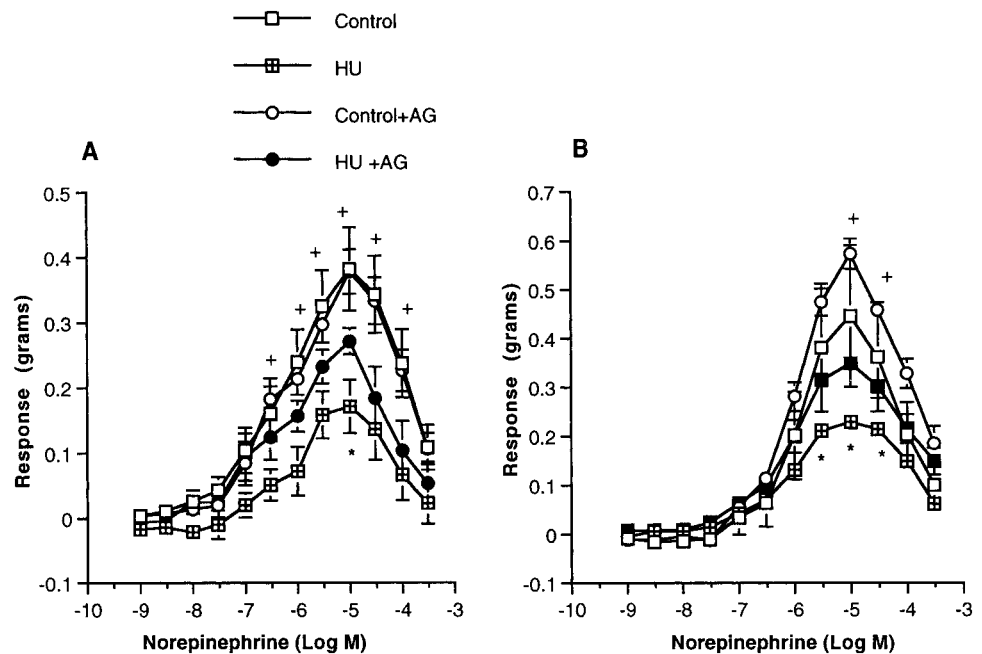


Fig. 10. Concentration-response curves for contractile effects of NE, in absence of L-arginine, in carotid artery (A, $n = 10$ rats) and femoral artery (B, $n = 5$ rats) rings with and without AG (1×10^{-4} M), from control and 20-day HU rats. Values are means \pm SE. *HU different from HU + AG ($P < 0.05$); + HU different from control ($P < 0.05$).

(Fig. 4). Sodium nitroprusside causes relaxation by a nitric oxide mechanism that is independent of the endothelium (16). The lack of a difference between control and HU carotid artery sensitivities to sodium nitroprusside suggests that HU did not alter the sensitivity of vascular smooth muscle to nitric oxide. Thus the greater sensitivity to ACh points to an HU effect that would manifest as an increase in the affinity of ACh for endothelial muscarinic receptors or an increase in eNOS activity or protein mass level activated by ACh. A preliminary study in the authors' laboratory using Western blot analysis indicated an increase in eNOS protein mass level in HU carotid artery (26). The reversal of the HU-induced vascular hyporesponsiveness caused by endothelium removal could be explained if it were found that HU increased eNOS activity or enzyme level. In this case, the endothelium

of the HU carotid artery could synthesize and release higher levels of nitric oxide, either basal or agonist stimulated. In turn, these higher levels of nitric oxide could exert a vasodilator effect that would attenuate the contractile response of the carotid artery to NE.

The effect of endothelium removal on HU-mediated depression of contractile response to NE in the femoral artery could not be adequately assessed in the present study. However, there were no differences in the sensitivities of control and HU femoral artery rings to the relaxing effects of ACh or sodium nitroprusside. Thus these results are consistent with the possibility that endothelial mechanisms contribute little to the HU effect in this vessel.

It has been reported by others (17, 18, 21), as well as by the present authors (11, 24, 33–35), that iNOS exists constitutively in vascular and nonvascular tissues. Moreover, iNOS expression can be altered by various disease states. Experiments carried out to explore the effect of HU on iNOS levels in the abdominal aorta and carotid and femoral arteries also provided insight into the presence of this enzyme in control tissues. iNOS synthesizes nitric oxide continuously in the presence of the substrate L-arginine (20). If iNOS is present in the blood vessels studied, then addition of exogenous L-arginine to the bathing medium should induce concentration-dependent relaxations in control and HU abdominal aorta and femoral and carotid artery rings precontracted with NE. Moreover, in each of the tissues the magnitude of relaxation was greater after HU. In the HU carotid artery the selective iNOS inhibitor aminoguanidine reversed only the relaxation induced by 1 μ M L-arginine, but in the HU femoral artery it reversed relaxation induced by 0.3 and 1 μ M L-arginine. Aminoguanidine had no effect on L-arginine-induced relax-

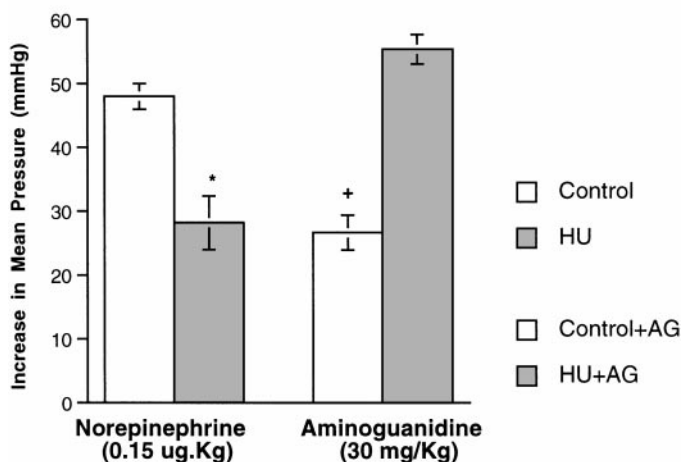


Fig. 11. Changes in mean blood pressure in control ($n = 5$) and HU ($n = 6$) rats in response to NE (0.15 μ g/kg iv) and AG (30 mg/kg iv). Values are means \pm SE; $n = 5$ rats. *Significantly different from control ($P < 0.05$); + significantly different from HU + AG response ($P < 0.05$).

ations in control or HU abdominal aorta rings. These results indicate the presence of iNOS in control and HU femoral and carotid artery rings. However, the level of iNOS seems to be upregulated to a greater extent in HU femoral artery rings than in HU carotid artery rings, as indicated by the reversal of 0.3 and 1 μM L-arginine-induced relaxations by aminoguanidine in the HU femoral artery rings. Also, the femoral artery, in general, seems to possess a higher level of iNOS than the carotid artery, since aminoguanidine significantly reversed the relaxation caused by 1 μM L-arginine in the control femoral artery but not in the control carotid artery.

To explore this phenomenon further, femoral artery rings were exposed to L-arginine, and NE CRCs were obtained in the presence and absence of aminoguanidine. Aminoguanidine had no effect on the control vessels but completely reversed the depressed contraction to NE in the HU vessels. These experiments were based on the following assumptions. In a vessel ring exhibiting upregulation of iNOS, addition of L-arginine would result in a sustained production of nitric oxide, since the activity of iNOS is known to be largely dependent on availability of the substrate. In turn, this endogenous vasodilator (nitric oxide) would attenuate contractile responses to NE. Addition of aminoguanidine would block iNOS and, therefore, nitric oxide production, restoring the full capacity of the vessel to contract to NE. That aminoguanidine fully restored the level of contraction of HU rings to that of the control rings argues that HU had upregulated iNOS in the femoral artery.

NE CRCs were also obtained in the abdominal aorta and carotid artery rings in the presence of L-arginine and with and without aminoguanidine. In these vessels, aminoguanidine had no effect on the contractions of control or HU vessels to NE; i.e., the HU contraction was depressed and remained depressed in the presence of aminoguanidine. These results combined with the results in Fig. 8 suggest that control and HU carotid artery rings are likely to possess lower levels of iNOS, whereas control and HU abdominal aorta rings lack iNOS compared with control and HU femoral artery rings.

NE CRCs were also obtained in carotid and femoral artery rings, without addition of exogenous L-arginine, in the presence and absence of aminoguanidine. In this case, aminoguanidine caused a slight increase in the NE contractile response in both blood vessels from HU, but not control, rats. The small, aminoguanidine-induced increase in NE contractile response in the HU vessels was significant at only one concentration of NE (10 μM) in the carotid artery and at three concentrations of NE (1–30 μM) in the femoral artery. These results confirm that iNOS is present in both blood vessels but suggest that relatively little endogenous L-arginine is available in these blood vessels under the *in vitro* conditions of the experiments. These experiments imply that other mechanisms, in addition to an increase in iNOS, contribute to the HU-induced hypore-

sponsiveness to NE observed *in vitro* in the carotid and femoral arteries. We suggest that the experiments that provide the most information concerning the magnitude of the differences in iNOS content/activity between carotid and femoral arteries are those in which NE CRCs were obtained in the presence of exogenous L-arginine (see above).

The present experiments suggest that HU increased the expression and/or activity of iNOS in the femoral artery and, to a lesser extent, in the carotid artery. It is not known whether HU increases iNOS expression more extensively in the vasculature. If such increased expression were more widespread, for example, throughout the hindlimb vascular bed, this could have profound hemodynamic consequences. To explore this possibility, an *in vivo* experiment was carried out to determine whether manipulation of iNOS function could impact blood pressure responses. First, NE was injected, producing a rapid, transient blood pressure peak. The magnitude of blood pressure elevation caused by NE in the HU rats was approximately one-half that observed in the control animals. This *in vivo* hyporesponsiveness to NE suggested that the vascular effect of HU is sufficiently generalized to influence total peripheral resistance and, therefore, blood pressure. Next, aminoguanidine was injected to explore the effect of iNOS inhibition. The blood pressure elevation caused by aminoguanidine in the HU rats was more than double that in the control animals. The following factors are likely responsible for blood pressure elevation elicited by aminoguanidine. First, iNOS must be present in the vasculature. In addition, endogenous L-arginine must be available. If these two conditions are met, iNOS would synthesize nitric oxide continuously, bathing the vasculature in this vasodilator. In turn, nitric oxide from this source would be one of several factors operating together to determine arterial pressure. One possible explanation for the weaker blood pressure response to NE in the HU rats could be that the vasculature of this model was bathed in more nitric oxide and, therefore, was less capable of responding with vasoconstriction to NE. Support for this theory comes from the more than two times greater blood pressure elevation caused by iNOS inhibition in HU rats with aminoguanidine. The implication of the larger effect of aminoguanidine in HU rats is that iNOS activity, namely, nitric oxide synthesis, was making a larger contribution to the resting blood pressure in this model than in the control. Because this contribution is in the direction of vasodilation and blood pressure lowering, blockade of iNOS would be expected to produce a greater blood pressure elevation in HU and did so. The weaker blood pressure response to NE, combined with the stronger response to aminoguanidine in HU rats, strongly suggests that vascular iNOS expression and/or activity was greater in HU than in control rats.

Further support for the interpretation of our *in vivo* results comes from a study of the pressor effect of aminoguanidine in control rats vs. those receiving

lipopolysaccharide (LPS) 3 h before aminoguanidine injection (38). Aminoguanidine increased blood pressure modestly in control animals but caused a more than twofold greater blood pressure elevation in the LPS-treated rats. LPS was shown to increase iNOS activity more than fivefold in the lungs of the treated rats compared with controls. The authors suggested that a similar increase in vascular iNOS could underlie their blood pressure observation (38).

It is known that the nonselective NOS inhibitor L-NAME elevates blood pressure, in part, by a central action. L-NAME blocks neuronal NOS (nNOS), thereby inhibiting central sympathetic outflow (4). It is possible that a similar mechanism could have mediated the in vivo blood pressure effects of aminoguanidine observed in the present study. Although the present experiments do not rule this out, it seems unlikely. Wolffe and Lubeskie (37) showed that aminoguanidine is 50–500 times more selective for iNOS than for nNOS. Thus we suggest that the blood pressure-elevating action of aminoguanidine was mediated by iNOS inhibition in the vasculature. Future experiments will have to resolve this issue.

Numerous investigators (6, 25, 27) have used the HU model to simulate cardiovascular deconditioning in rats. A study by Murison (23) argued that the effects of HU are not a result of an increase in stress due to HU treatment. They reported initial increases in adrenal weight and plasma corticosterone levels at the onset of HU, but these levels returned to control levels as the animals adapted to the HU. In addition, the absence of cardiac hypertrophy suggests that the cardiovascular system is not stressed by HU. Finally, Kahwaji and co-workers (14) reported that although HU reduces the maximal vasoconstrictor response to NE, it has no effect on the response to serotonin. This also argues against a nonspecific effect of HU, such as stress. Taken together, these findings suggest that HU induces vascular changes via hemodynamic effects rather than a stress response caused by HU. On the other hand, the present results do not rule out the possibility that the placement of a harness on the tail of the rat to achieve HU could have an effect itself, independent of the HU-induced hemodynamic effects. Future experiments comparing harnessed rats in the hindlimb-elevated position with harnessed rats in the horizontal position will be required to resolve this issue.

In conclusion, the present results suggest that HU in the rat may have the following consequences: endothelial vasodilator mechanisms may be predominantly increased in the carotid artery and, possibly, related vascular beds in the upper half of the body, except the cerebrovasculature (2, 9). In contrast, iNOS expression/activity may be increased in the femoral artery and, possibly, related vascular beds in the lower half of the body. The consequence of these HU-mediated changes may be a sustained elevation of vascular nitric oxide levels. If true, the sustained exposure to elevated levels of the endogenous vasodilator, nitric oxide, could be one of the factors underlying the previously shown (25) vascular hyporesponsiveness to NE.

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